

REMARKS

The above amendment adds no new matter and is merely made to more accurately describe the invention.

It is respectfully submitted that the application is now in condition for allowance, which allowance is respectfully requested.

The Commissioner is authorized to charge any fee or credit any overpayment in connection with this communication to our Deposit Account No. 11-1449.

Respectfully submitted,

KOHN & ASSOCIATES




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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as "First Class Mail" on August 13, 2001 and is addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on August 13, 2001.


Marie M. DeWitt



VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

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Mammalian two-hybrid assay. Full-length cDNAs encoding human [CAM-1, RSV-F and RSV-G were amplified from mRNA of RSV infected HEp-2 cells using gene specific primers, each of which included an appropriate restriction site for subsequent cloning [ICAM-1: Forward-5'CCT GGC GAA TTC CAG ACA TCT GTG TCC CCC TCA (SEQ ID No: 1), Reverse - 5'GTG TGG ATC CAC TGC CAC CAA TAT (SEQ ID No: 2); F gene: Forward- 5' CAA GAA TTC ATG GAG TTG CTA ATC CTC AAA CA (SEQ ID No: 3), Reverse- 5' CTA TGT CGA CTT AGT TAC TAA ATG CAA TAT TAT TTA (SEQ ID No: 4); and G gene: Forward- 5' AAT GAA TTC ATG TCC AAA AAC AAG GAC CAA CGC (SEQ ID No: 5), Reverse- 5'GTT GTC GAC TAA CTA CTG GCG TGG TGT GTT (SEQ ID No: 6)]. The ICAM-1 cDNA was cloned in-frame with the activation domain (AD) derived from VP16 protein of herpes simplex virus in the vector pVP16 (Clontech, Palo Alto, CA). The cDNA encoding F or G protein was amplified, and cloned in frame to the GAL4 DNA-binding domain (DBD) of the pM vector. The orientation and the reading frame of all these fusion constructs were verified to be correct by restriction endonuclease analysis. The pG5CAT vector was used to detect protein-protein interaction by expression of CAT enzyme. CAT activity was assayed using ELISA (Boehringer Mannheim, Indianapolis, IN) following the manufacturer's protocol. The vectors, pM, pVP16 and pG5CAT, were transfected into NIH 3T3 fibroblast cells to determine the basal level of CAT activity.

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Reverse transcriptase PCR analysis. The total RNA was isolated from the harvested cells and tissue samples using Trizol (Life Tech., Gaithersburg, MD). Random primed cDNA was prepared using superscript II RNase H- reverse transcriptase (Life Tech., Gaithersburg, MD). The first strand cDNA product (1 μ l) was amplified using *Taq* polymerase (Life Tech., Gaithersburg, MD). Forward and reverse primers used are as follows: RSV-N forward: 5'-GCG ATG TCT AGG TTA GGA AGA A-3' (SEQ ID No: 7); reverse: 5'-GCT ATG TCC TTG GGT AGT AAG CCT-3' (SEQ ID No: 8) (Vignola et al., 1993); ICAM-1 forward: 5'-ATG GCT CCC AGC AGC CCC-3' (SEQ ID No: 9); reverse: 5'-CAC CTG GCA GCG TAG GGT-3' (SEQ ID No: 10) and β -actin forward: 5'-CGC GAG AAG ATG ACC CAG-3' (SEQ ID No: 11); reverse: 5'-ATC ACG ATG CCA GTC GTA C-3' (SEQ ID No: 12). ALL PCR reactions were denatured at 95°C for 1', annealed at 56°C for 1' and extended at 72°C for 1 minute for 40 cycles. All amplifications were RNA specific, as no bands were seen in the control (no template) PCR samples. The reaction products were separated on 1.5% agarose gels.